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In this proposal, we hypothesize that inhibition of IGF action by IGFBP-1 will prevent breast cancer in a SV40 Tag transgenic model of breast cancer. We will test this hypothesis with two specific aims: 1) to inhibit IGF action at the mammary epithelial cell by creating transgenic mice that express IGFBP-1 under the control of the whey acidic protein (WAP) promoter and 2) to test the ability of IGFBP-1 to suppress tumorigenesis by mating these animals with C3/Tag transgenic mice. To date, we have generated two founder lines containing the IGFBP-1 transgene and several F1 and F2 animals were analyzed. Unfortunately, while the transgene was clearly integrated into these animals, we were unable to detect expression of IGFBP-1 protein. We also generated animals with an insulator sequences. Despite defining 7 additional founder lines, we were unable to demonstrate expression of IGFBP-1 mRNA or protein.

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Introduction

Insulin-like growth factor-I (IGF-I) action is required for normal mammary gland development in the rodent (1). In model systems, IGF action is also required for malignant transformation of a variety of oncogenes (2). Since IGF signaling also affects estrogen receptor function (3), there is a strong rationale for blocking IGF action as a way to prevent breast cancer. Since we had previously shown that IGF binding protein-1 (IGFBP-1) expression in breast cancer cells renders them refractory to IGF action (4), it is possible that ectopic IGFBP-1 expression in the mammary gland could also block IGF signaling and affect breast cancer development. The purpose of this project was to create transgenic mice expressing IGFBP-1 in the mammary gland. We hope overexpression of this binding protein can neutralize IGF action and inhibit breast cancer development.

Body

Specific Aim (Task) #1 - To inhibit IGF action at the mammary epithelial cell by creating transgenic mice that express IGFBP-1 under the control of the whey acidic protein (WAP) promoter

a. Months 0-3 - Create WAP-IGFBP-1 transgene vector
b. Months 3-9 - Create and identify IGFBP-1 F1 progeny
c. Months 9-16 - Characterize level of IGFBP-1 expression in mammary gland, determine influence of IGFBP-1 expression on lactation, examine activation of IGFR1

As noted in previous progress reports, we were unable to generate mice that express IGFBP-1 in the mammary gland. In our first attempts, we examined mammary gland and milk for IGFBP-1 protein. We also evaluated mammary gland for IGFBP-1 mRNA. Unfortunately, we were unable to document expression of either mRNA or protein in the mammary gland. We felt that we had selected too few founders to identify animals with expression of the protein. These results are discussed in the September 2002 Progress report.

In order to address this concern, we decided to re-inject animals with the original WAP-IGFBP-1 vector. Our collaborator (Dr. David Largaespada) reviewed our progress and suggested that addition of an insulator sequence may potentially enhance the ability of the transgene to be expressed. With Dr. Largaespada's assistance, we created a new transgene construct bearing the insulator sequence as outlined in the 2003 Progress Report.

As noted in the 2003 Progress Report, we had a setback in the generation of these animals as our specific pathogen free (SPF) animal facility suffered an outbreak of pinworms and mouse hepatitis virus. Our Animal Use Committee decided to completely close the facility, transfer the animals to the animal facility in a newly constructed building, sterilize the old facility, then re-open the transgenic core facility for investigator use.

These outbreaks in the SPF facility essentially delayed this work for 12 months as the transgenic facility was closed for that period of time. Thus, in 2003, we asked for a no-cost extension.

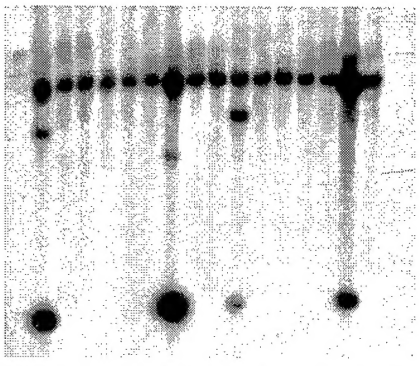

Figure 1 – Southern Blot of Founders

At the time of the 2003, we were able to generate several founder lines (a representative Southern blot is shown in Figure 1). To address the reviewer's previous concern, we have not used PCR to screen for founders. We have only examined tail DNA by Southern blot. In total, we generated an additional 10 founder lines of the WAP promoter construct and 7 founder lines of the WAP-insulator construct.

We generated numerous F2 crosses. We sampled milk and resected glands for isolation of mRNA. To examine milk and extracts from mammary glands, we performed IGFBP-1 immunoblotting, IGF ligand blotting, and IGFBP-1 ELISA. To look for mRNA expression, we used ribonuclease protection assays. Despite these intensive efforts to detect IGFBP-1 mRNAs or transcripts, we still have not been able to document IGFBP-1 protein or mRNA expression in any of these animals.

Key Research Accomplishments

- Generated insulator constructs and injected into oocytes
- Generated 17 founder animals
- Generated approximately 298 animals
- Examined mammary gland for IGFBP-1 mRNA and protein expression from approximately 40 animals

Reportable outcomes

Given the necessity to generate new founder animals, we do not yet have reportable outcomes.

Conclusions

Even with the setback from contamination of our animal SPF facility, we still have not been able to generate animals with IGFBP-1 mRNA and protein expression. As noted above in Figure 1, we have had no difficulty in identifying animals with transgene expression. Despite this success, mRNA and protein was undetectable. As noted in the earlier progress report, there may be several reasons for this failure. First, my colleagues who work in mouse mammary models have had a similar unfortunate experience where a gene will not express in the mammary gland despite integration of the transgene. It is possible that IGF signaling is required for mammary gland function, and IGFBP-1 expressing cells are selected against.

Second, a different mammary gland "specific" promoter may allow expression. We have used the whey acidic protein promoter, and it is possible that the MMTV promoter may have been a better choice. Of course this has been a frustrating project. However, we are committed to generating animals with IGFBP-1 expression in the mammary gland. While we have exhausted the support from this proposal, we are exploring the possibility that MMTV-IGFBP-1 promoter may allow for IGFBP-1 expression in the mammary gland. We have performed one more oocyte injection and will pursue this project using other funding sources.

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Appendices - None